COMPARATIVE CHEMOSENSITIVITY TO AMINO ACIDS AND THEIR ROLE IN THE FEEDING ACTIVITY OF BATHYPELAGIC AND LITTORAL CRUSTACEANS [1]

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At the greater ocean depths, animals seek their prey in the virtual absence of light. These predators must therefore use other sensory stimuli to locate prey. It is likely their faculties of chemoreception and mechanoreception are of particular importance. This study attempts to clarify the role of chemoreception in the feeding activity of deeper-living crustaceans by comparing the chemosensitivity of a bathypelagic species with that of a shallower-living benthopelagic species and three intertidal species. The adopted approach examines the behavioral and electrophysiological responses of these species to a range of amino acid concentrations in an attempt to determine the thresholds of these responses.

The bathypelagic species chosen is the lophogastrid mysid *Gnathophausia ingens,* a predator of small fish and crustaceans which inhabits depths of 600–750 meters by day, spreading at night to depths of about 400–900 meters (Childress, 1969). At lesser depths, the galatheid crab *Pleuroncodes planipes* functions both as an epipelagic filter feeder and a benthic species of the continental shelf, at maximum depths of around 300 meters (Boyd, 1963). Intertidal species are represented by *Pagurus hirsutiusculus,* an anomuran crab, *Spirontocaris taylori,* a carid shrimp, and *Cancer antennarius,* a brachyuran crab.

Amino acid sensitivity in littoral crustaceans and the stimulation of feeding activity by amino acids has been demonstrated in previous investigations. Receptor thresholds of 5×10^{-5} M glutamic acid in *Carcinus maenas* dactyls (Case and Gwilliam, 1961) and 5×10^{-7} M taurine in *Homarus americanus* antennules (Ache, 1972) have been reported. Levandowsky and Hodgson (1965) reported *Panulirus argus* to initiate feeding in the presence of 5×10^{-4} M glutamic acid. This study examines chemoreceptive acuity in additional littoral species to provide a broader context in which to view the chemoreceptive abilities of deeper-living species.

An earlier investigation of receptor specificity in the *Panulirus interruptus* antennule (Fuzessery, unpublished) is included in this study as a partial basis for the choice of constituents of the amino acid mixture used as a feeding stimulant. Two general classes of receptors were found which responded maximally to either mono- or dicarboxylic amino acids, implying that a combination of these compounds would have an increased effect on feeding behavior through maximal activation of receptors with different specificities. Ache (1972) and Shepheard (1974)

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also found receptor specificity to different amino acids in the antennule receptors of *Homarus americanus.* McLeese (1970), and Shelton and Mackie (1971) have provided behavioral evidence that maximal feeding stimulation in crustaceans requires combinations of compounds, although these studies included other organic compounds in addition to amino acids.

Finally, we will attempt to place the results of this study into the context of the bathypelagic environment of *G. ingens,* and examine the possible means of employment of the chemoreceptive faculties of this species in its feeding activity.

MATERIALS AND METHODS

Gnathophausia ingens was collected with a midwater trawl from basins off the coast of southern California. *Pleuroncodes planipes* was collected with a meter net at shallower depths off Baja California. The three littoral species tested were collected in tidepools on the Santa Barbara coast.

G. ingens is one of the few bathypelagic species suitable for this study, as it has been maintained in the laboratory for up to 2.5 years (Childress, 1971).

Behavioral studies

The standard feeding stimulant used in this study was an equimolar solution of L-glutamic acid, taurine and DL alpha amino-n-butyric acid. This mixture was chosen for the following reasons: (1) studies by other investigators (Hodgson, 1958; Case and Gwilliam, 1961; Case, 1964; Laverack, 1964; Levandowsky and Hodgson, 1965; and Ache, 1972) have shown these compounds are effective feeding stimulants in crustaceans, (2) a mixture rather than a single amino acid was considered to more closely approximate a situation naturally encountered by crustaceans, and (3) an earlier study of amino acid specificity in *P. interruptus* antennules indicated the presence of two classes of receptors responding maximally to either mono- (*e.g.,* aminobutyric acid) or dicarboxylic (*e.g.,* glutamine acid) amino acids (see results). The remaining mixture component, taurine, activated both receptor types and was found to be particularly stimulatory in this and other studies (Case, 1964; Ache, 1972; Shepheard, 1974). Natural feeding stimulants (food extracts) were not used because of obvious difficulties in identifying constituents and quantifying their concentrations.

The feeding responses of the five species tested were initially observed in the presence of 10^{-3} M amino acid mixture for the purpose of describing response characteristics. These responses were categorized in a hierarchy of response intensity, and a minimal criterion for a feeding response exhibition was defined for each species. Behavioral sensitivity to the mixture was determined by observing the percentage of individuals exhibiting a feeding response in each of a range of mixture concentrations.

For the tests, individuals were placed in separate chambers containing filtered seawater. One hundred and fifty specimens of *G. ingens* and 175 specimens of *P. hirsutiusculus* were tested in 250 ml glass bowls; tests on *G. ingens* were conducted in a temperature-controlled room at 7° C, the normal ambient temperature of this species. Sixteen specimens of *S. taylori* and 90 specimens of *P. planipes* were tested in 1000 ml glass bowls. *Spirontocaris taylori* required a small clump

of red algae in which to "conceal" itself; without this, this species would circle the bowl continuously. Fifteen specimens of *C. antennarius* were tested in 3 gal. aquaria; all individuals of this species had a carapace width of less than 4 inches. At least 30 min were allowed for the individuals to achieve a quiescent state (*i.e.,* no avoidance responses and a minimum of exploratory behavior) before testing. Solutions were introduced *via* syringe through air tubing at a rate of 0.5–1.0 ml/sec. An inital seawater introduction, serving both to determine the individual's response to mechanostimulation and as an attempt to adapt-out this response, was followed by either a second seawater introduction serving as control or a given concentration of the mixture. The individual was considered sensitive to the test solution if the designated feeding response was observed between the time of introduction and one minute following. The effect of chemical stimulation on a given species was considered to be the percentage of individuals responding to a given concentration minus the percentage responding to the seawater control.

In the same manner, the responses of *G. ingens* were also observed in the presence of additional individual amino acids, representing structural groups not included in the mixture, L-phenylalanine, L-arginine, and hydroxyproline, and to trimethylamine hydrochloride and betaine hydrochloride. The responses of *P. hirsutiusculus* to the individual components of the mixture were observed to determine the relative stimulatory effects of single and combined amino acids. Finally, the effect of simultaneous chemo-tactile stimulation was tested in *G. ingens* by touching blotter paper permeated with a given mixture concentration to the thoracic legs for one second.

With the exception of *G. ingens*, individuals were not without food more than five days before testing. All tests on *G. ingens* were done within 10 days of capture, during which time they were not fed.

Electrophysiological studies

The response thresholds of the receptors of *G. ingens* dactyls and antenna, and *C. antennarius* dactyls and third maxillipeds were examined electrophysiologically for comparison with the behaviorally determined thresholds of these species. *Panulirus interruptus* antennular receptors in both the medial and lateral flagella were examined for specificities to different amino acids, but thresholds were not investigated. Sensory activity was recorded from subdivided axon bundles dissected from ablated appendages, using chlorided silver wire electrodes in conjunction with standard recording instrumentation. Responses from *P. interruptus* antennules were recorded on kymograph film; all other receptor activity was integrated *via* a strip-chart recorder. Appendages were situated in a trough isolated from the exposed fibers by a rubber dam. Exposed fibers were submerged in filtered sea water; appendges were kept moist by periodic washes. Test solutions were applied to the appendages *via* syringe through a 20 gauge hypodermic needle.

In the *P. interruptus* specificity investigation, the introduction of the various amino acids (0.01 M) was interspersed by seawater washes to reduce receptor adaptation. The first amino acid tested was reintroduced at the end of the test series to compare response similarities. A loss of sensitivity to the concluding amino acid invalidated the series. Response intensities were calculated by counting the filmed impulses.

TABLE I

Percent of individuals responding to amino acid mixture.

	Con-trol	Concentration (M)											
		10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	10^{-10}	10^{-11}	10^{-12}	10^{-13}
Pagurus hirsutiusculus	(13) 8	(48) 98	(20) 80	(67) 55	(24) 42	(15) 33	(32) 16	—	—	—	—	—	—
Spirontocaris taylori	(18) 16	—	—	(18) 95	(30) 80	(12) 67	(12) 58	(12) 42	—	—	—	—	—
Gnatho-phausia ingens	(53) 9	(49) 72	(48) 43	(40) 44	(18) 44	(16) 31	(18) 44	(18) 33	(34) 38	—	(18) 11	—	—
G. ingens chemo-tactile response	(54) 48	(18) 100	(18) 72	—	—	(18) 67	(18) 61	—	—	—	—	—	—
Cancer antennarius	(31) 13	—	(16) 100	(27) 89	(19) 84	(27) 64	(15) 73	(8) 63	(8) 88	(8) 38	(8) 50	—	—
Pleuroncodes planipes	(32) 13	—	—	(15) 100	(31) 97	(16) 69	(31) 61	—	(23) 43	—	(16) 44	—	(22) 18

(X) = Number of tests.   (—) = No tests.

In the threshold studies, a series of amino acid mixture concentrations was introduced, beginning with the lowest concentration. As fiber bundles were rarely subdivided to single receptor units, all chemoreceptive activity was judged against mechanoreceptive activity evoked by sea water. Response intensities were calculated by manually planing the area beneath the integrated response curves during the first 10 seconds. The mechanoreceptive activity of a given preparation was subtracted from the chemoreceptive activity.

RESULTS

Chemoreceptive acuity based on behavioral criteria

For each of the five species tested, feeding responses elicited by the mixture are listed below in the approximate sequence in which they occur, beginning with responses of least intensity and progressing to those which more clearly indicate awareness of a chemical presence representing potential food. Variations of these sequences occurred, as when in the presence of a high concentration, a response of greater intensity was immediately elicited, rather than a progression. For each species the response or combinations of responses judged to be a minimum criterion of a feeding response is stated. These responses represent apparent attempts to secure or ingest food simulated by amino acids. The percentage of individuals exhibiting a feeding response at each range of mixture concentrations is presented in Table I and Figure 3. Also tabulated are the frequencies of response of *G. ingens* to additional individual amino acids and related compounds (Table II) and the responses of this species to chemo-tactile stimulation (Table I).

Feeding responses in the presence of amino acid mixture

Gnathophausia ingens. (1) Anterior endopodites are moved in a circular motion over the mouth. This motion appears to be related to filter feeding. (2)

TABLE II

Percentage of Gnathophausia ingens *individuals responding to amino acids and related compounds.*

Compound	Molarity	N	% Responding
Betaine hydrochloride	10^{-4}	17	88
	10^{-6}	17	65
L-Arginine	10^{-3}	34	65
	10^{-5}	34	44
Hydroxyproline	10^{-3}	17	59
	10^{-5}	17	35
Phenylalanine	10^{-3}	17	41
	10^{-5}	17	35
Trimethlamine oxide	10^{-3}	17	29
	10^{-5}	17	24
Control	—	53	9

Endopodites are partially extended in an unconcerted manner. (3) Endopodites are extended approximately perpendicular from the body in a concerted motion, in the manner of reaching. (4) The abdomen is arched ventrally, either perpendicular to the body or with the telson almost in contact with the mouth. This is perhaps a method of entrapping prey against the ventral body surface. A feeding response was defined as (3) a concerted endopodite extension.

Pleuroncodes planipes. (1) Antennae are oriented toward the stimulus, and the antennule flicking rate is increased. (2) The third pair of maxillipeds are rubbed together. (3) The third pair of maxillipeds are outstretched and brought back to the mouth. This is probably filter feeding activity. (4) The chelipeds are brought to the mouth. (5) The substrate is prodded with the chelipeds or periopodites. (6) A capture response is executed in which the periopodites and/or chelipeds are brought to the ventral body surface in the manner of entrapment. A feeding response was defined as (4) as the motion of bringing the chelipeds to the mouth.

Cancer antennarius. (1) Antennal orientation motion and antennule flicking rate is increased. (2) Periopodites are extended in the direction of the stimulus and/or used to prod the substrate, sometimes followed by a slow search. (3) The third maxillipeds are flared and set into a lateral motion. (4) The chelipeds are brought to the mouth. (5) A capture response is executed involving grabbing and/or pouncing upon suspected prey and entrapping against the ventral body surface. A feeding response was defined as (3) maxilliped activity in conjunction with cheliped activity and/or a capture response. Maxilliped activity alone did not constitute a feeding response.

Spirontocaris taylori. (1) Antennal motion is increased, and the antennules are erected and their flicking rate increased. (2) The third maxillipeds are rubbed together. (3) The substrate is prodded with the periopodites, with these legs moving rapidly up and down in a characteristic asynchronous fashion. (4)

There is an increase in the intensity of (3), with the shrimp leaving its original position in a slow search. A feeding response was defined as (3) the rapid up and down motion of the periopodites.

Pagurus hirsutiusculus. (1) Antennal motion and antennular flicking rate is increased. (2) The third maxillipeds are extended. (3) The substrate is prodded with the periopodites and/or chelipeds. (4) The chelipeds are brought to the mouth. (5) A capture response is executed involving rapid cheliped extension, scissoring or the cheliped pincers, and grabbing motion. A feeding response was defined as (4) the motion of bringing the chelipeds to the mouth.

The same behavioral criteria applied to the additional tests on *G. ingens* in response to additional individual amino acids and related compounds, and to chemo-tactile stimulation. They also applied to tests on *P. hirsutiusculus* responses to the individual components of the amino acid mixture.

Feeding response characteristics were the same in all species to both the amino acid mixture and actual food. That these species demonstrated feeding behavior to amino acids in the absence of visual cues indicates a high degree of chemical control over this activity. Even in orientation of actual food, visual cues appear to play a comparatively minor role. Only *S. taylori*, an active intertidal shrimp, demonstrated visual orientation by occasionally intercepting pieces of food as they sank from the surface. The most commonly observed orientation to food in the species examined was a slow search apparently governed by chemical cues and effected by leg extension and, in the benthic species, prodding of the substrate in the general direction of the food.

Electrophysiological results

Panulirus interruptus—receptor specificity. Antennular receptors in *P. interruptus* demonstrated a specificity to different amino acids. Different preparations responded maximally to either monocarboxylic (*e.g.*, glycine and DL-alpha amino-

TABLE III

Percent of Pagurus hirsutiusculus *individuals responding to amino acid mixture and individual mixture components.*

	Molarity	N	% Responding
Mixture	1.5×10^{-2}	48	98
	1.5×10^{-4}	67	55
Taurine	1.5×10^{-2}	32	88
	1.5×10^{-4}	32	44
Glutamic Acid	1.5×10^{-2}	31	65
	1.5×10^{-4}	30	23
Aminobutyric Acid	1.5×10^{-2}	30	67
	1.5×10^{-4}	31	19
Control	—	13	8

n-butyric acid) or dicarboxylic (*e.g.,* L-glutamic and L-aspartic acid) amino acids, indicating the presence of at least two general classes of receptors. Responses to both groups of amino acids in a given preparation was more commonly encountered, but these responses generally appeared to be the product of a greater number of receptor units in the recorded population, implying that both receptor types may have been present, or that there are also receptors with broader specificities. Of 19 preparations selected for consistency of response to given amino acids throughout a test series, 11 responded maximally to either mono- or dicarboxylates. All 11 are medial flagellum preparations. Figure 1 represents the average number of impulses per second elicited by four preparations responding maximally to monocarboxylates (A) and seven to dicarboxylates (B). The averages are a pooling of responses to glycine and DL-alpha amino-n-butyric acid, and to L-aspartic acid and L-glutamic acid. As also indicated (Fig. 1A and B), taurine, a sulfur-substituted amino acid, activated both receptor types but appeared to be more stimulatory to receptors responding maximally to dicarboxylates. Figure 2 illustrates examples of recordings from two antennular preparations from which these data were calculated.

Gnathophausia ingens—chemoreceptive acuity. Dactyl and antennal receptors were tested for responses to mixture concentrations ranging from 1.5×10^{-2} M to 5×10^{-10} M. The response threshold of dactyl receptors was 6×10^{-8} M, that of the antennae 5×10^{-7} M. In both appendages, response intensity and duration increased with concentration. Responses to 10^{-6} M generally adapted within 15
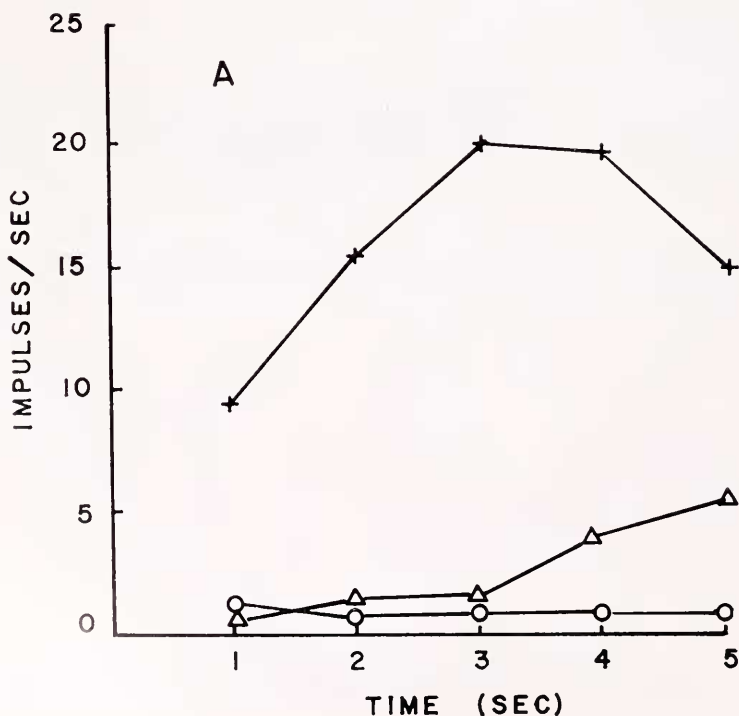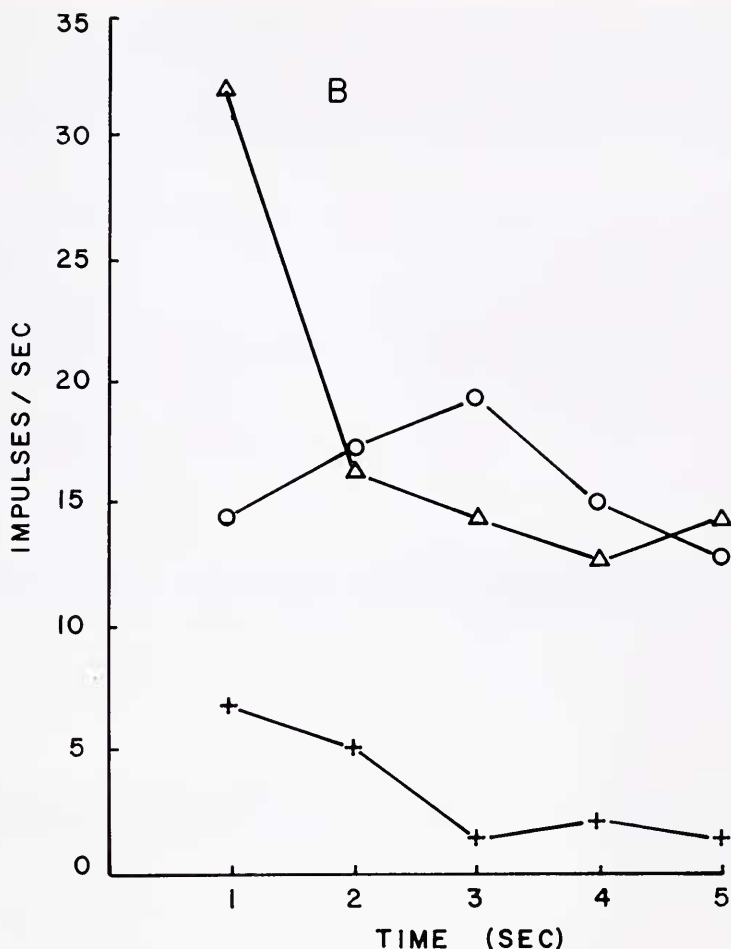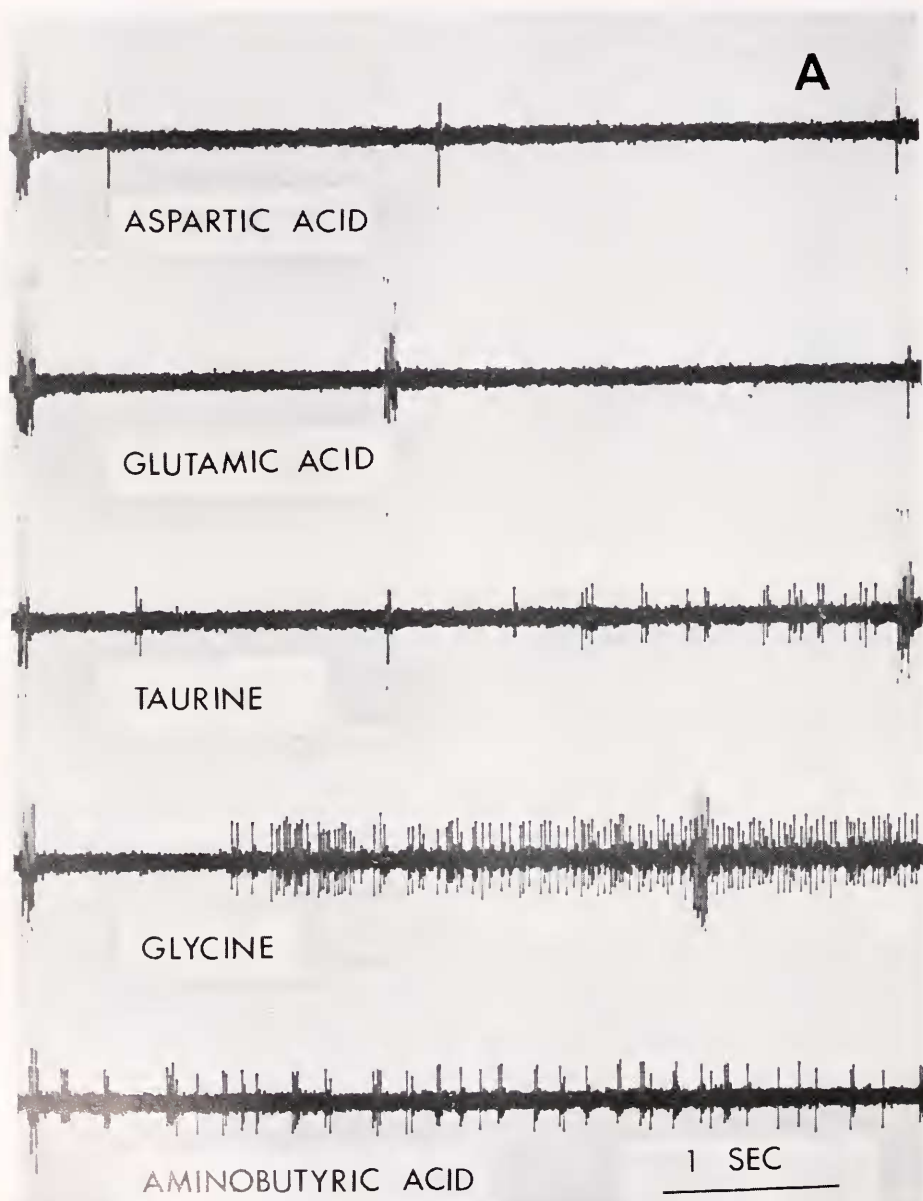
FIGURE 1. Receptor specificity in *P. interruptus* antennule. Symbols represent an averaging of responses to glycine and DL alpha amino-n-butyric acid (cross), L-aspartic acid and L-glutamic acid (open circles), and taurine (open triangles). Four preparations responded primarily to monocarboxylates (A), and seven to dicarboxylates (B). Taurine was more stimulatory in (B). Stimulus is one drop of 0.01 M of a given amino acid.

seconds; responses to 10^{-2} M lasted up to one minute. Laverack (1964) and Ache (1972) reported antennule receptor responses of similar duration to high amino acid concentrations in *Panulirus argus* and *Homarus americanus*. Response latencies were not critically examined and were often masked by mechanoreceptive activity, but in general, activity ensued within 0.1 seconds of stimulus introduction. Chemoresponses were reversible by washing the preparation with sea water.

Cancer antennarius—chemoreceptive acuity. The response threshold of both dactyl and maxilliped receptors was 5×10^{-5} M of the mixture. Response characteristics were similar to those reported by Case (1964) in an extensive study on

the dactyl receptors of this species, except that responses of greater duration elicited by high concentrations were occasionally recorded. Response intensity generally peaked within one second of stimulus introduction and usually decayed rapidly in comparison to the more tonic receptor responses of *G. ingens*. Responses to one drop of 10^{-3} M of the mixture generally extinguished within 5 seconds, while re-
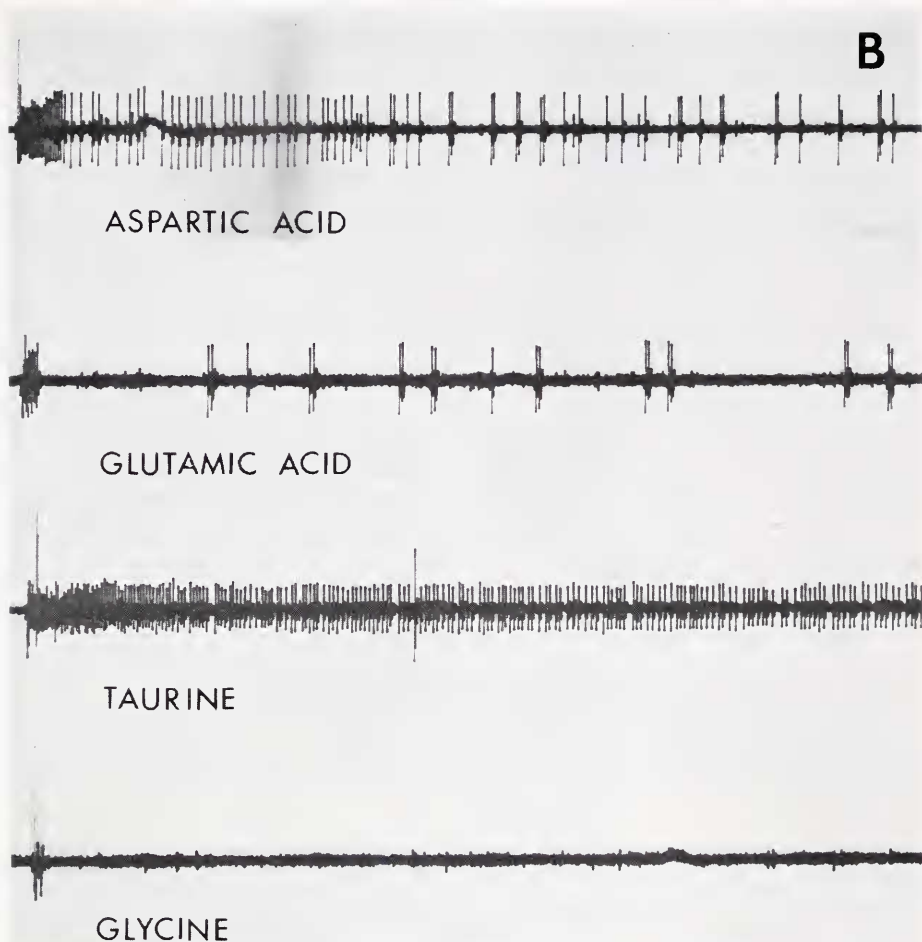
FIGURE 2. Two examples of receptor specificity in the *P. interruptus* antennule from which the preceding graphs (Fig. 1) were obtained. Responses are from receptors of the medial flagellum. (A) shows specificity to monocarboxylates, (B) to dicarboxylates. Note strong taurine response in (B). Point of stimulation indicated by initial phasic mechanoresponse.

sponses to higher concentrations persisted at lower activity levels for up to 30 seconds, though infrequently these responses to high concentrations (10^{-2} M) lasted up to two minutes. Response time and intensity increased with concentration (Fig. 4). All chemoreceptive activity was reversible by seawater washes.

DISCUSSION

The presence of amino acid receptors in crustaceans and the initiation of feeding activity by amino acids has been reported in previous studies, but the chemoreceptive acuities of crustaceans and the high degree of chemical control over their
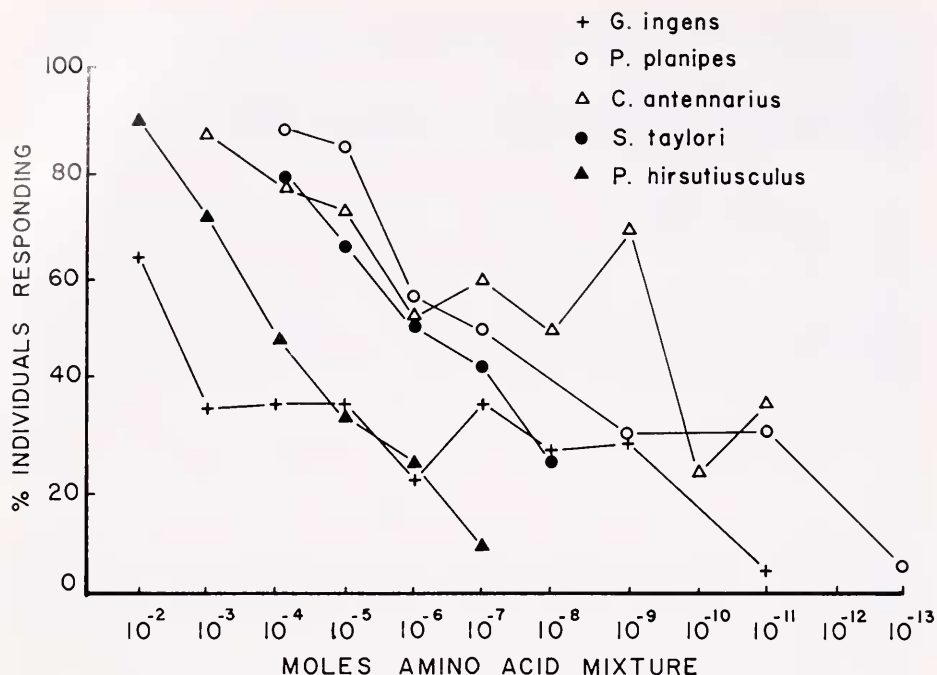
FIGURE 3. The percentage of individuals of the five species exhibiting a feeding response in the presence of a range of amino acid mixture concentrations. For each species, the percent responding to the seawater control is subtracted from the percent responding to the mixture (see Table I).

feeding behavior has not been adequately documented. This study attempts to clarify this aspect of crustacean behavior by investigating amino acid feeding response thresholds in five species from a spectrum of marine habitats. The low amino acid concentrations (10^{-7} to 10^{-12} M) at which feeding responses were exhibited show not only a higher degree of chemosensitivity than has been previously demonstrated, but also indicates that chemical cues may play a dominant role in the feeding activity of crustaceans in general, at least among the totally aquatic species. This high degree of chemical control is strikingly apparent when one observes a crab execute a capture response upon food simulated by as little as 10^{-9} M of amino acid, in the complete absence of visual cues. At higher amino acid concentrations (10^{-2} to 10^{-4} M), the feeding responses appear to occur almost as a reflex.

The greatest behavioral acuity was demonstrated by the two deeper-living species, *G. ingens* and *P. planipes,* and the predatory littoral crab, *C. antennarius.* In *P. planipes* and *C. antennarius* the amino acid mixture evoked feeding activity at minimum concentrations of 10^{-11} to 10^{-12} M, while the behavioral threshold of *G. ingens,* 10^{-10} to 10^{-11} M, was slightly higher. The two remaining intertidal species, *S. taylori* and *P. hirsutiusculus,* demonstrated thresholds of 10^{-8} to 10^{-9} M and 10^{-6} to 10^{-7} M amino acid mixture respectively. For comparison, pre-
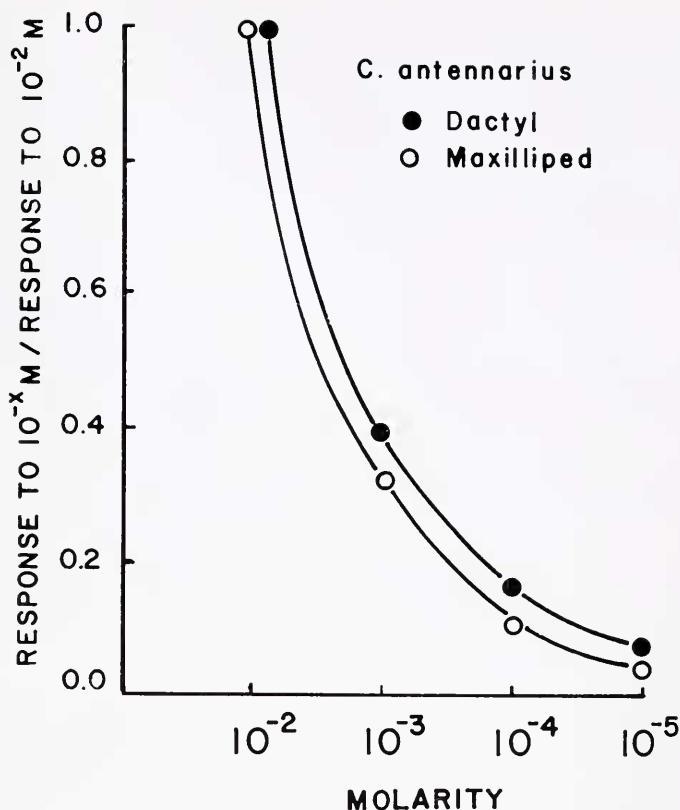
FIGURE 4. The average relative responses of 13 dactyl and 8 maxilliped preparations to a range of amino acid mixture concentrations. Receptor response to 10^{-2} M is arbitrary unity. Response intensity calculated from first 10 seconds of response.

vious behavioral studies have shown *Panulirus argus* to initiate feeding activity in the presence of 10^{-4} M amino acid (Levandowsky and Hodgson, 1965), and *Homarus americanus* to initiate food-search activity in the presence of 10^{-6} M amino acid (McLeese, 1970).

The electrophysiological thresholds for dactyl and third maxilliped receptors of *C. antennarius* were 10^{-5} M amino acid mixture. This is in agrement with the sensitivity found in the dactyl receptors of another brachyuran crab, *Carcinus maenas* (Case and Gwilliam, 1961). The dactyl receptor threshold of G. ingens (10^{-8} M amino acid mixture) is the lowest threshold reported in crustacean receptors, approximating both the antennal receptor threshold of this species (10^{-7} M amino acid mixture) and the antennular receptor threshold of 10^{-7} M amino acid reported in *Homarus americanus* (Ache, 1972; Shepheard, 1974). It has been postulated that, among the decapods, the dactyls, with lower chemosensitivity, function as contact chemoreceptors, while the more chemically sensitive antennules serve as distance receptors (Shepheard, 1974). Judging from the high chemo-

sensitivity of the dactyl receptors of *G. ingens,* this functional dichotomy is apparently not as distinct in this bathypelagic mysid.

Similar receptor sensitivities to amino acids have been reported in fish. Recording of olfactory lobe activity in *Ictalurus catus* (Suzuki and Tucker, 1971) and *Salmo gairdneri* (Hara, 1973) showed these species possess receptors activiated by amino acid concentrations between 10^{-7} and 10^{-8} M.

The discrepancies in behavioral and electrophysiological thresholds in *G. ingens* and particularly *C. antennarius* are probably due to two factors: technical difficulties involved in isolating single receptor unit fibers, allowing the possibility that other afferent activity (mechanorceptive and proprioreceptive) masked chemoresponses at lower concentrations; and behavioral responses utilized the entire receptor population of a species, possibly including more sensitive receptors not examined electrophysiologically. In *C. antennarius,* antennule receptors were not examined electrophysiologically because of the small size and delicate nature of these append- ages. Antennule receptors may have been responsible for the lower behavioral threshold observed in this species.

Another factor to be considered is that, of the species tested, *G. ingens* was the least suitable for behavioral study. This entirely pelagic species normally functions at great depths in essentially unlimited amounts of space, and is thus unaccustomed to the vertical and horizontal barriers imposed by captivity. Because this aspect of its environment was virtually impossible to simulate, this species, though an active feeder in captivity, was unable to exhibit orientation responses under test conditions. Therefore, only the most obvious feeding display (co- ordinated extension of the endopodites) was available as a test criterion. The unnatural environment may have served to raise the behavioral threshold of this species, particularily in light of the dactyl receptor responses to concentrations 1000 times more dilute than the *C. antennarius* dactyl threshold.

Finally, the behavioral acuities described do not represent the absolute receptor thresholds of these species, but rather are the amino acid concentrations that con- tained sufficient information about potential food, upon integration in the central nervous system, to stimulate the animal to feed. Furthermore, the defined feeding responses are direct attempts to secure food simulated by amino acids, and are thus conservative indices of the awareness of a chemical presence. While orientation responses, such as increased antennule flicking, also indicate the awareness of a chemical presence and occurred with greater frequency and at lower concentra- tions, the defined feeding responses were chosen because they provided less ambiguity.

The mixture of amino acids used in this study may have had a more stimulatory effect on feeding activity than single amino acids. *Panulirus interruptus* antennule receptors demonstrated some degree of specificity to either mono- or dicarboxylic amino acids, implying that a combination of these compounds could have an additive stimulatory effect (independent of concentration) by activating more receptor types, while single amino acids or mixtures of closely related compounds would be likely to compete for receptor sites. In an extensive study of responses to a spectrum of amino acids and other organic compounds, Shepheard (1974) similarly described two general classes of receptors in the antennules of *H. americanus* which responded maximally to either L. glutamic acid or hydroxy

L-proline. Laverack (1964) and Ache (1972) also reported amino acid specificity in crustacean antennule receptors, but, as in this study, non-specific activation was more commonly encountered, implying that either both receptor types were present in the recorded population, or that there are also receptors with broader specificity. An increased stimulatory effect of the mixture was also supported behaviorally in *P. hisutiusculus,* in which the mixture was more stimulatory than equal concentrations of the individual components (Table III). McLeese (1970), and Shelton and Mackie (1971) have also reported that crustaceans are maximally stimulated by combinations of compounds.

Another aspect of the *P. interruptus* antennule study which warrants attention is that the receptor responses to amino acids were recorded from receptors of the inner or medial flagellum of the antennule. In previous studies involving similar species (Laverack, 1964; Levandowsky and Hodgson, 1965; Ache, 1972; Shepheard, 1974) chemoreceptor activity has been reported only in the outer or lateral flagellum, which has a hair tuft encompassing aesthetasc hairs, lacking in the medial flagellum. If the medial flagellum possesses receptors activated by food odors (amino acids), it is possible the elaborate hair tuft of the lateral flagellum has a more specialized function, such as pheromone reception, in addition to amino acid reception.

Possible mechanisms involved in orientation to chemical cues may be inferred from the results of this study in conjunction with the work of previous investigators. We wish to briefly discuss four which warrant further investigation: quantitative discrimination, qualitative discriminaton, contact between chemoreceptive appendages in general, and contact between thoracic appendages and mouthparts.

The direct relationship between stimulus concentration and receptor response intensity, also noted by Case (1964) and Ache (1972), would potentially provide the ability to orient up a chemical gradient in a food search. The apparent discrimination of various amino acids would afford qualitative analysis of chemical feeding stimulants and support Shepheard's (1974) contention that crustaceans may distinguish these compounds from one another.

Contact between chemoreceptive appendages (brushing or combing one appendage with another) following the introduction of chemical stimulants was noted in the five species examined behaviorally. This "preening" activity occurred in such forms as rubbing maxillipeds together, rubbing periopodites together, or combing antennae or antennules with maxillipeds, periopodites or chelipeds. Because this activity occurs primarily among chemoreceptive appendages following reception of chemical cues, and because it appears to be divorced from direct procurement of food, it possibly serves orientation through enhancement of chemical reception. While this activity may simply represent a mechanical cleaning of receptor sites, unpublished results found by Lindsey (1975, University of California) indicate the mechanoreceptive activity, as would be induced by contact between appendages, may actually influence chemoreceptor activity at the neuronal level.

A more specific form of appendage contact with perhaps more obvious implications is the action of placing the chelipeds to the mouth, as noted in three test species. This may simply represent direct attempts to ingest food simulated by amino acids. However, the particularily habitual nature of this activity in *P.*

hirsutiusculus, which executes this activity with rapidity and regularity when chemically stimulated, suggests this behavior may serve to present potential food or food odors to receptors at the mouth for verification of proper chemical cues before actual feeding is initiated. This mechanism has not been studied in crustaceans, but Atema (1971) reported that in catfish (*Ictalurus natilis*) deinnervation of chemoreceptors on the mouth blocked acceptance of food, even though intact external receptors enabled the fish to locate the food. Perhaps a similar dichotomy of function exists in crustaceans.

Focusing on *G. ingens* and the utilization of high chemosensitivity in the bathypelagic environment, Vinogradov (1970) has suggested that the two primary food sources of species below depths of 500 meters would be sinking zooplankton remains and vertical migrators transporting the resources of the more productive surface waters downward to non-migrators, such as *G. ingens.* This implies that deeper-living pelagic species would be scavengers or filter feeders of zooplankton remains and/or carnivores preying directly or indirectly upon vertical migrators. Calman (1909) suggested that *G. ingens* is a coarse filter feeder based on the existence of "feeding currents" produced by the maxillipeds. Our observation of the modification of the hairs of the anterior endopodites into sieve-like structures and the circular motion of these appendages over the mouth in the presence of amino acids supports this hypothesis. However, such factors as gut contents, fecal material, rapacious feeding upon fish caught in the same trawl, and apparent capacity through size and mobility (it is the largest entirely pelagic crustacean) indicate that *G. ingens* preys upon crustaceans and small fish of the midwater. This species may therefore function both as a scavenger and predator, consuming any usable organic material it encounters.

The high chemoreceptive abilities and the hypothesized mechanism of orienting up a chemical gradient may serve to detect prey through leakage of these compounds, possibly facilitated by tissue damage or through normal waste excretion. A particularly high percentage of individuals responded to betaine (Table II), a methylated amino acid form associated with protein synthesis and breakdown, found in the blood and excretory products of both vertebrates and invertebrates (Kutscher and Ackerman, 1933). Betaine would also be expected in decomposing zooplankton. The high frequency of feeding response exhibition to tactile stimulation in the absence of amino acids (48%) indicates mechanoreception is also an important feeding cue. In the absence of light, a food search initiated by dilute chemical cues would be effectively executed by the antennae, which are longer than the body and extend laterally and back, and by the antennular rami, one of which extends forward, the other laterally and ventrally. These appendages would thus monitor three axes of the immediate search area.

In conclusion, the bathypelagic mysid *G. ingens* is among the more chemosensitive of the species examined, and this capacity is probably of primary importance in locating food in an essentially aphotic environment. However, we have observed that intertidal species also exhibit feeding behavior in the presence of low amino acid concentrations in the absence of visual cues. It is likely there is a high degree of chemical control over the feeding behavior of aquatic crustaceans in general. Visual cues appear to generally play a comparatively minor role, even in the shallower-living species, so the absence of light may not pose as significant an orientation problem to bathypelagic crustaceans as might be assumed.

SUMMARY

1. Feeding response exhibition in five marine crustaceans was observed in the presence of a range of concentrations of an amino acid mixture composed of equal parts DL alpha amino-n-butyric acid, L-glutamic acid and taurine. *Gnathophausia ingens*, *Pleuroncodes planipes*, and *Cancer antennarius* displayed behavioral thresholds of between 10^{-10} and 10^{-12} M; *Spirontocaris taylori* and *Pagurus hirsutiusculus* displayed thresholds between 10^{-7} and 10^{-9} M.

2. Electrophysiological response thresholds to the amino acid mixture were examined in *G. ingens* and *C. antennarius*. The antennular and dactyl receptors of *G. ingens* displayed thresholds of 10^{-7} and 10^{-8} M respectively. Maxilliped and dactyl receptors of *C. antennarius* both displayed a threshold of 5×10^{-5} M.

3. *Panulirus interruptus* antennule receptors displayed a degree of specificity to mono- and dicarboxylic amino acids.

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